A/RE

DOCKET NO. : GLIS-0064 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Reissue Application of:

Buhr, *et al.*

U.S. Patent No.: 5,672,697 **Issued: September 30, 1997**

Serial No.: 652,978 **Group Art Unit: 1623***

Filing Date: February 8, 1991 **Examiner: G. Kunz***

For: NUCLEOSIDE 5'-METHYLENE PHOSPHONATES

EXPRESS MAIL LABEL NO: EL40122685US
DATE OF DEPOSIT: September 29, 1999

Assistant Commissioner for Patents
Washington DC 20231

Sir:

REISSUE APPLICATION TRANSMITTAL LETTER

Transmitted herewith is the application for reissue of U.S. Patent No. **5,672,697,** issued on **September 30, 1997,** of **Chris Buhr, Mark Matteuicci, Norbert W. Bischofberger, and Brian Froehler,** entitled **NUCLEOSIDE 5'-METHYLENE PHOSPHONATES.**

Enclosed are the following:

1. SPECIFICATION, CLAIM(S) AND DRAWING(S)

☒ 39 page(s) of specification

☒ 5 page(s) of claims

☒ 1 page(s) of abstract

Note: This must include the entire specification and claims of the patent, with the matter to be omitted by reissue enclosed in square brackets; and any additions made by the reissue must be underlined, so that the old and new specifications and claims may be readily compared. Claims should not be renumbered and the numbering of claims added by reissue should follow the number of the highest numbered patent claim.

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*Enter the Group Art Unit and Examiner from which the original patent was issued.

☐ sheet(s) of ☐ formal/☐ informal drawings.

☐ No changes in the drawings upon which the original patent was issued are to be made. Therefore, in accordance with 37 C.F.R. § 1.174, please find attached, in the size required for original drawings:

☐ a copy of the printed drawings of the patent.

☐ a photoprint of the original drawings.

2. DECLARATION AND POWER OF ATTORNEY

☒ 8 Pages of declaration and power of attorney.

☐ An Associate Power of Attorney.

3. PRELIMINARY AMENDMENT (check if applicable)

☐ enclosed herewith.

4. OFFER TO SURRENDER THE ORIGINAL LETTERS PATENT IN ACCORDANCE WITH 37 C.F.R. § 1.178 IS ATTACHED

☒ Offer to surrender is by the inventor.

☒ along with assent of assignee.

☐ Offer to surrender is by the assignee of the entire interest (and the reissue application does not seek to enlarge the claims of the original patent).

5. LETTERS PATENT

☐ Original letters patent attached.

☐ Declaration that original letters patent lost or inaccessible.

☒ Original letters patent or declaration that original letters patent lost or inaccessible will be submitted after prosecution on the merits but before the application has been allowed.

Note: "The application may be accepted for examination in the absence of the original patent or the declaration but one or the other must be supplied before the case is allowed." 37 C.F.R. § 1.178.

Note: "If a reissue be refused, the original patent will be returned to applicant upon his request." 37 C.F.R. § 1.178.

6. CONSENT OF ASSIGNEE AND ASSIGNEE'S INTEREST

In accordance with 37 C.F.R. §1.171(a), this application for reissue is accompanied by Consent of Assignee for Reissue and Assignee's Statement of Ownership Interest [37 C.F.R. §3.73(b)] with:

☐ Copy or copies of recorded documentary evidence of a chain of title from the original owner to the assignee.

☒ Designation by reel and frame where documentary evidence of a chain of title from the original owner to the assignee is recorded in the Office.

7. INFORMATION DISCLOSURE STATEMENT (check if applicable)

☐ attached.

8. PRIORITY - 35 U.S.C. § 119

☐ Priority of application Serial No. @@ filed on @@ in @@ (country) is claimed under 35 U.S.C. § 119.

☐ The certified copy has been filed in prior application Serial No. @@ filed on @@

9. FEE CALCULATION (37 C.F.R. §§ 1.16(h), (i) and (j))

			SMALL ENTITY		NOT SMALL ENTITY	
			RATE	FEE	RATE	FEE
			$380.00	$	$760.00	$
	No. Filed	No. Extra				
TOTAL CLAIMS	26- 20 OR the number of claims in original patent, whichever is greater =	6	$9 each	$	$18 each	$108.0
INDEP. CLAIMS	minus number of independent claims in original patent =	9	$39 each	$	$78 each	$702.00
MULTIPLE DEPENDENT CLAIMS ARE TREATED AS ORDINARY CLAIMS FOR FEE PURPOSES. 37 C.F.R. §1.16(j).						
TOTAL FILING FEE DUE				$		$1,570.00

10. SMALL ENTITY STATUS (if applicable)

Note: A new verified statement is required for the reissue even if one has been filed in the original patent.

☐ A Verified Statement Claiming Small Entity Status under 37 C.F.R. §§ 1.9 and 1.27 is enclosed.

11. METHOD OF PAYMENT OF FEES

☒ A check in the amount of $1,570.00 is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

☐ Please charge my Deposit Account No. 23-3050 in the amount of $.

12. AUTHORIZATION TO CHARGE ADDITIONAL FEES

☒ The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 23-3050.

☒ Any additional filing fees required under 37 C.F.R. §§ 1.16(a), (f) or (g) (filing fees) including fees for presentation of extra claims (37 C.F.R. §§ 1.16(b), (c) and (d)).

☒ Any additional patent application processing fees under 37 C.F.R. § 1.17 and under 37 C.F.R. § 1.20(d).

☒ The Commissioner is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 23-3050.

☒ Any patent application processing fees under 37 C.F.R. § 1.17 and under 37 C.F.R. § 1.20(d).

☐ The issue fee set in 37 C.F.R. § 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b).

☒ Any filing fees under 37 C.F.R. § 1.16 including fees for presentation of extra claims.

This sheet is attached in duplicate.

Date:September 29, 1999

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For: NUCLEOSIDE 5'-METHYLENE PHOSPHONATES

Assistant Commissioner for Patents
Washington DC 20231

Sir:

CONSENT OF ASSIGNEE FOR REISSUE
and
ASSIGNEE'S STATEMENT OF OWNERSHIP INTEREST IN REISSUE[1]

I. In accordance with 37 CFR §1.172(a), **ISIS Pharmaceuticals, Inc.**, assignee of the entire interest in U.S. Patent No. **5,672,697**, granted on **September 30, 1997,** to inventor(s) **Chris Buhr, Mark Matteucci, Norbert W. Bischofberger, and Brian Froehler,** hereby consents to reissue of said patent for the reasons set forth in the accompanying Reissue Declaration.

II. In accordance with 37 CFR §1.172(a), said assignee of the entire interest in United States Patent No. **5,672,697**, hereby establishes assignee's ownership of said patent and its right to take action therein under 37 CFR §3.73(b) by:

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* Enter the Group Art Group and Examiner from which the original patent was issued.

[1] This paper must accompany the application for reissue.

☒ specifying that evidence of said ownership is recorded in the Office for each assignment in the chain of title at Reel <u>5666</u> , Frame <u>0340</u> and at Reel <u>9833</u>, Frame <u>0012</u>.

[Note: list reel and frame for each assignment in chain]

☒ enclosing herewith copies of executed assignment(s) dated **<u>February 28, 1991, March 20, 1991 and March 18, 1999</u>** which have been submitted for recording in the Office.

[Note: if other documentation is necessary to show ownership, the Office may require recordation thereof under 37 CFR §2.73(b).]

Date: <u>9/29/99</u>

ISIS PHARMACEUTICALS, INC.

By: ______________________
B. Lynne Parshall, Esq.
Executive Vice President and
Chief Financial Officer

NUCLEOSIDE 5'-METHYLENE PHOSPHONATES

FIELD OF THE INVENTION

This invention relates to novel methylene phosphonate nucleosides which exhibit antiviral and antitumor activity and novel oligonucleotides derived from methylene phosphonate nucleoside monomers that have enhanced nuclease stability. The invention also relates to processes for preparing the novel compounds, their derivatives and oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages. The oligonucleotides are resistant to nuclease degradation and are useful for diagnostic and therapeutic applications.

BACKGROUND ART

Antisense and triple helix oligonucleotides are synthetic oligonucleotides which bind complementary nucleic adds (*i.e.*, sense strand RNA or duplex DNA sequences) via hydrogen bonding, thereby inhibiting expression of these sequences. Therapeutic intervention at the nucleic acid level using oligonucleotides offers a number of advantages. Inhibition of gene expression is more efficient than inhibition of the protein encoded by the gene since transcription of a single DNA sequence gives rise to multiple copies of mRNA which, in turn, are translated into many protein molecules.

Oligonucleotides have been used to inhibit gene expression in a variety of systems. There are several reviews that discuss this topic.[1-4] In addition, the use of oligonucleotides in sequence-specific recognition of double stranded DNA[5,6] as well as potential chemotherapeutic agents,[7] has been reviewed.

An important feature of the antisense oligomeric probes is the design of the backbone of the administered oligomer. Specifically, the backbone should contain internucleoside linkages that are stable in vitro and should be structured such that the oligomer is resistant to endogenous nucleases, such as nucleases that attack the phosphodiester linkage.[8] At the same time, the oligomer must also retain its ability to hybridize to the target DNA or RNA. In order to ensure these properties, a number of modified oligonucleotides have been constructed which contain alternate internucleoside linkages. Several of these oligonucleotides are described in Uhlmann, E. and Peyman, A., Chemical Reviews (1990) 90:543-584. Among these are methylphosphonates (wherein one of the phosphorous-linked oxygens has been replaced by methyl); phosphorothioates[8,9] (wherein sulphur replaces one of these oxygens) and various amidates (wherein NH_2 or an organic amine derivative, such as morpholidates or piperazidates, replace an oxygen). These substitutions confer enhanced stability, for the most part, but suffer from the drawback that they result in a chiral phosphorous in the linkage, thus leading to the formation of 2^n diastereomers where n is the number of modified diester linkages in the oligomer. The presence of these multiple diastereomers considerably weakens the capability of the modified oligonucleotide to hybridize to target sequences. Some of these substitutions also retain the ability to support a negative charge and the presence of charged groups decreases the ability of the compounds to penetrate cell membranes. There are numerous other disadvantages associated with these modified linkages, depending on the precise nature of the linkage. Phosphorodithioate modified backbones have been made.[9,10] These modified oligonucleotides are nuclease resistant and are diastereomerically pure. However, these modifications further reduce the affinity of the oligonucleotide for its intended target.[10c] A variety of modified nonionic[11] oligonucleotides including methylphosphonate, phosphoroamidate, and phosphotriesters generally are either composed of a mixture of diastereomers, have a low affinity for intended targets, or both.

A deoxyoligonucleotide comprised from nucleotide monomers that contain a methylene (--CH_2 --) group substituted for the 5'-oxygen may be resistant to nucleases, especially those that leave a 3'-phosphate moiety after cleavage of the internucleotidic bond. This results from the fact that the requisite P--C bond can not be cleaved under normal physiological conditions. Additionally, a single diastereomerically pure deoxyoligonucleotide could be prepared, as the internucleotide phosphorous linkages would be achiral. We refer to the nucleotides containing a methylene (--CH_2 --) group substituted for the 5'-oxygen as 5'-methylene phosphonates.

The preparation of ribo (*i.e.*, 2'-OH) 5'-methylene phosphonates is well documented in the literature.[12] Uridine,[13-15] adenosine,[13-15] and guanosine [16] 5'-methylene phosphonates have been prepared. A number of analogues of adenosine 5'-methylene phosphonate have been prepared.[17-23] In addition, ribavirin 5'-methylene phosphonate,[24] as well as a ribo 5'-methylene phosphonate containing thiazole-4-carboxamide as the base, has been prepared.[25] Ribo compounds having a 3'-methylene phosphonate have also been prepared.[26-28]

There are very few reports of 2'-deoxy 5'-methylene phosphonates in the literature, and these are all related to thymidine. Only the syntheses of 5'-methylene phosphonates of thymidine,[29] 3'-azidothymidine (AZT),[30,31] and 2'-deoxy-5-fluoro-uridine[32] have been reported. There have been no reports on the syntheses of 5'-methylene phosphonates derived from 2'-deoxyadenosine, 2'-deoxycytidine, or 2'-deoxyguanosine. There also have been no reports on the synthesis of 5' methylene phosphonate nucleosides having 5-iodouracil, 2-aminopurine or 2,6-diaminopurine as the base. The 5-iodouridine 5' methylene phosphonate compound would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from thymidine as described for compounds 33 and 37 below. The 2-aminopurine and 2,6-diaminopurine nucleoside 5' methylene phosphonates would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from deoxyadenosine as described for compounds 36 and 40 below.

Several ribo 5'-methylene phosphonate dimers have been synthesized. These include UpCH_2 U and UpCH_2 A.[33,34] Several ribo 3'-methylene phosphonate dimers,[33] as well as a trimer[28] have been synthesized. These ribo dimers and trimer were prepared using the diester method

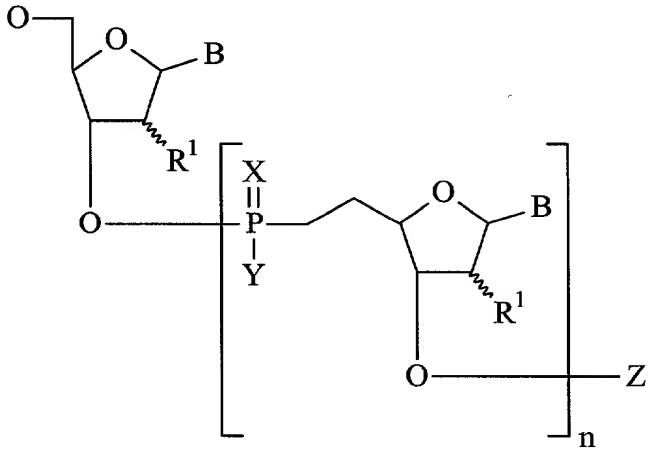
of oligonucleotide synthesis.[35,36] This method suffers from low product yields, and difficulties in purification of the final product.[35,36] The method is generally not useful in the preparation of longer oligonucleotides. Recently, a ribo oligonucleotide 10-mer consisting of 5'-methylene phosphonates, $ApA(pCH_2A)_8$, was prepared enzymatically using polynucleotide phosphorylase.[37] This technique, however, cannot be used for the preparation of oligonucleotides having a defined sequence of mixed bases.

Only one 2'-deoxy dimer, $TpCH_2$ T, and one 2'-deoxy trimer, $TpCH_2$ $TpCH_2$ T, have been reported in the literature.[29] Only the 5'-methylene phosphonate derived from thymidine was used in the dimer and trimer. No mixed base 2'-deoxy 5'-methylene phosphonate dimers or longer mixed base, 2'-deoxy 5'-methylene phosphonate oligonucleotides have been reported. Additionally, no 2'-deoxy 5'-methylene phosphonate oligonucleotides longer than a 3-mer of any kind have been reported. However, recently the synthesis of oligodeoxynucleotides containing 5'-methylene phosphonates of 2'-deoxy-4'-carbocyclic nucleosides has been reported W. Frick and S. W. Schneller, Meetings Abstract, Conference on Nucleic Acid Therapeutics, Jan. 13-17, 1991, Clearwater Beach, Fla., p63).

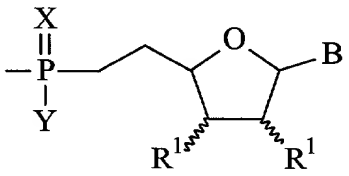
The present invention relates to the synthesis of 2'-deoxy-5'-methylene phosphonate oligonucleotides of length 2-30 of mixed base composition. These oligodeoxynucleotides are prepared using the phosphotriester method [38] from suitably protected 2'-deoxy 5'-methylene phosphonate nucleotide monomers. We prepared novel 5'-methylene phosphonates in both a protected form that was suitable for oligonucleotide synthesis, as well as in a completely deprotected form. Some of the novel 5'-methylene phosphonates that were prepared were derived from 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyguanosine. The monomers described herein are suitable for solid phase oligonucleotide synthesis by triester chemistry. Previous methods utilized diester chemistry which is more difficult and generates low yields of product. Oligonucleotides containing 2'-deoxy-2'-fluoro-ribonucleotides are of interest because the conformation of the sugar closely resembles that of RNA and consequently these oligonucleotides have a higher affinity to DNA than normal oligodeoxyribonucleotides (M. Ikehara, Heterocycles 1984, 21,

DISCLOSURE OF THE INVENTION

The present invention discloses oligonucleotides and methods for their synthesis of formula (I):



and stereoisomers thereof, wherein each B is independently a purine or pyrimidine base or modified form each Z is independently a noninterfering substituent, preferably hydrogen, PO_3^- or a protecting group; each R^1 is independently hydrogen, hydroxyl, fluorine or methyl ester; each Y is independently OR^2, $N(R^2)_2$ or SR^2 wherein, each R^2 is independently hydrogen or alkyl (1-12 C); X is selected from oxygen and sulfur; n is an integer from 1 to 200. Bases such as adenine, guanine, cytosine, thymine and uracil as well as modified forms (base analogs) such as 5-methylcytosine, aziridinylcytosine, 8-hydroxy-N^6-methyladenine, pseudoisocytosine and inosine are preferred. The oligonucleotides contain one or more 5' methylene phosphonate linkages. The oligonucleotides may be synthesized from derivatives disclosed herein of monomers of formula (II):



wherein B is a purine or pyrimidine base or modified form; each R^1 is independently hydrogen, hydroxyl, fluorine or methyl ester; each Y is independently OR^2, $N(R^2)_2$ or SR^2 wherein, each R^2 is independently hydrogen or alkyl (1-12 C); and X is selected from oxygen and sulfur. Bases such as guanine, adenine, cytosine, thymine, uracil, iodouracil,

8-hydroxy-N^6-methyladenine, aziridinylcytosine, 2-aminopurine, 2, 6-diaminopurine or other base analogs or altered forms may be utilized. Alternative monomer structures, such as 2',3' epoxides and 2',3'dideoxy didehydro sugars may also be synthesized.

The free 5'-methylene phosphonate nucleosides present enzymatically nonhydrolysable isosteres of mononucleotides. As such they can be converted intracellularly by cellular kinases to the corresponding nucleoside phosphono triphosphates, incorporated into DNA by polymerases and thus interfere with cellular metabolism. Thus, such nucleoside phosphonates potentially exhibit antiviral and antitumour activity. For example, several acyclic methylene phosphonates such as the methylene phosphonates derived from ganciclovir, and acyclovir are potent antivirals.[39-42] Other nucleoside phosphonates have been claimed in a patent application (Elmer Reist et al, Stanford Research Institute, PCT publication no. WO 84/04748). The novel 5'-methylene compounds that are described herein thus have useful antiviral or antitumour activities.

The oligonucleotide and nucleoside monomer compounds possess antiviral activity and can be used in the control or prevention of viral infections, e.g. of herpes simplex vital infections. The in vitro activity of the compounds of formula I and their tautomers in inhibiting herpes simplex virus type 2 (HSV-2) can be demonstrated by means of the following plaque reduction procedure. Host VERO cells are infected with virus stock containing a known number of infectious virions in the presence of various concentrations of compound. Plaques in the cell monolayer are then counted and compared to untreated controls and to acydovir treated controls. The degree of inhibition at each concentration of compound is expressed as a percentage of the control titer (100%). The IC_{50} value, namely the concentration of compound which inhibits viral activity by 50%, is then calculated. The results that are obtained with representative compounds show that virus titer reductions occur.

The compounds disclosed herein can be used as medicaments in the form of pharmaceutical preparations which contain them in association with a compatible pharmaceutical carrier material. This can be an organic or inorganic carrier suitable for enteral, e.g. oral, or parenteral administration. Examples of such carriers are water, gelatin, gum arabic, lactose, starch,

magnesium stearate, talc, vegetable oils, polyalkylene glycols and petroleum jelly. The pharmaceutical preparations can be made up in a solid form, e.g. as tablets, dragees, suppositories or capsules, or in a liquid form, e.g. as solutions, suspensions or emulsions; they may be subjected to standard pharmaceutical operations, e.g. sterilization and/or may contain adjuvants, e.g. preserving, stabilizing, wetting or emulsifying agents, salts for varying the osmotic pressure or buffers. The compounds may also be formulated in a manner suitable for administration as an aerosol. They may also contain other therapeutically valuable substances.

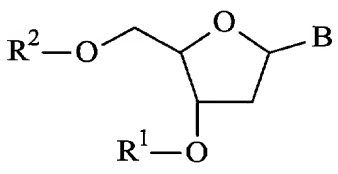
The compounds disclosed herein and their tautomers can be administered for the control or prevention of viral infection, such as herpes simplex viral infections, to warmblooded animals in need of such treatment. The disclosed compounds and their tautomers can be administered to adult humans in a daily dosage of from about 1 to 1000 mg, preferably about 5 to 500 mg. The daily dosage may be administered as a single dose or in divided doses. The above dosage range is given by way of example only and can be varied upwards or downwards depending on factors such as the particular compound being administered, the route of administration, the severity of the indication being treated and the condition of the patient.

Experimental Section

General. Flash chromatography refers to the procedure of Still et. al.[43] Drying refers to drying over Na_2SO_4, filtration, and concentration. All reactions requiring dry solvents were run under a dry argon atmosphere.

The following six tables show structures for compounds 1 through 90.

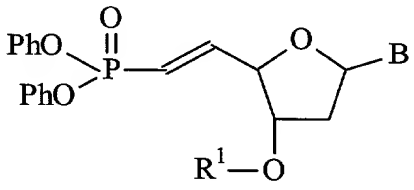
TABLE 1



Compound	B	R^1	R^2
1	G^{Ib}	H	H
3	C^{Bz}	H	H
5	A^{Bz}	H	H
2	G^{Ib}	H	DMT
4	C^{Bz}	H	DMT
6	A^{Bz}	H	DMT
7	T	H	DMT
8	G^{Ib}	TBS	H
9	C^{Bz}	TBS	H
10	A^{Bz}	TBS	H
11	T	TBS	H
12	T^{Bn}	Bn	H

For tables 1-6; G = guanine; C = cytosine; A = adenine; T = thymine; G^{Ib} = N^2 -isobutyrylguanine; C^{Bz} = N^4-benzoylcytosine; A^{Bz} = N^6 -benzoyladenine; T^{Bn} = N^3-benzylthymine; Bn = benzyl; DMT = 4,4'-dimethoxytrityl; TBS = t-butyldimethylsilyl; +HTEA = hydrogentriethylammonium

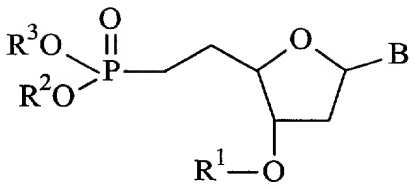
TABLE 2



Compound	B	R^1
13	G^{Ib}	TBS
15	C^{Bz}	TBS
17	A^{Bz}	TBS
19	T	TBS
21	T^{Bn}	Bn

For definition of abbreviations, see Table 1.

TABLE 3

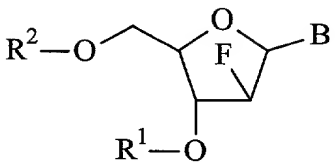


	Compound	B	R^1	R^2	R^3
5	14	G^{Ib}	TBS	Ph	Ph
	16	C^{Bz}	TBS	Ph	Ph
	18	A^{Bz}	TBS	Ph	Ph
	20	T	TBS	Ph	Ph
	22	T^{Bn}	Bn	Ph	Ph
10	23	T^{Bn}	Bn	Me	Me
	24	T^{Bn}	H	Me	Me
	25	T^{Bn}	Bn	Bn	Bn
	26	G^{Ib}	H	Ph	Ph
	27	C^{Bz}	H	Ph	Ph
15	28	A^{Bz}	H	Ph	Ph
	29	T	H	Ph	Ph
	30	G^{Ib}	H	Me	Me
	31	C^{Bz}	H	Me	Me
	32	A^{Bz}	H	Me	Me
20	33	T	H	Me	Me
	34	G^{Ib}	H	H	H
	35	C^{Bz}	H	H	H
	36	A^{Bz}	H	H	H
	37	T	H	H	H
25	38	G	H	H	H
	39	C	H	H	H

40	A	H	H	H
41	G^{Ib}	DMT	Ph	Ph
42	C^{Bz}	DMT	Ph	Ph
43	A^{Bz}	DMT	Ph	Ph
44	T	DMT	Ph	Ph
45	G^{Ib}	DMT	Ph	+HTEA
46	C^{Bz}	DMT	Ph	+HTEA
47	A^{Bz}	DMT	Ph	+HTEA
48	T	DMT	Ph	+HTEA

For definition of abbreviations, see Table 1.

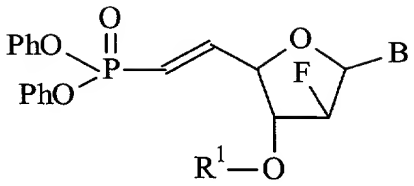
TABLE 4



Compound	B	R^1	R^2
49	G^{Ib}	H	H
51	C^{Bz}	H	H
53	A^{Bz}	H	H
50	G^{Ib}	H	DMT
52	C^{Bz}	H	DMT
54	A^{Bz}	H	DMT
55	T	H	DMT
56	G^{Ib}	TBS	H
57	C^{Bz}	TBS	H
58	A^{Bz}	TBS	H
59	T	TBS	H

For definition of abbreviations, see Table 1.

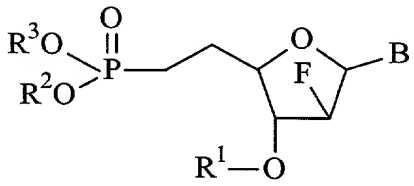
TABLE 5



Compound	B	R^1
60	G^{Ib}	TBS
62	C^{Bz}	TBS
64	A^{Bz}	TBS
66	T	TBS

For definition of abbreviations, see Table 1.

TABLE 6



Compound	B	R^1	R^2	R^3
61	G^{Ib}	TBS	Ph	Ph
63	C^{Bz}	TBS	Ph	Ph
65	A^{Bz}	TBS	Ph	Ph
67	T	TBS	Ph	Ph
68	G^{Ib}	H	Ph	Ph
69	C^{Bz}	H	Ph	Ph
70	A^{Bz}	H	Ph	Ph
71	T	H	Ph	Ph
72	G^{Ib}	H	Me	Me
73	C^{Bz}	H	Me	Me
74	A^{Bz}	H	Me	Me

	75	T	H	Me	Me
	76	G^{Ib}	H	H	H
	77	C^{Bz}	H	H	H
	78	A^{Bz}	H	H	H
	79	T	H	H	H
	80	G	H	H	H
	81	C	H	H	H
	82	A	H	H	H
	83	G^{Ib}	DMT	Ph	Ph
	84	C^{Bz}	DMT	Ph	Ph
	85	A^{Bz}	DMT	Ph	Ph
	86	T	DMT	Ph	Ph
	87	G^{Ib}	DMT	Ph	+HTEA
	88	C^{Bz}	DMT	Ph	+HTEA
	89	A^{Bz}	DMT	Ph	+HTEA
	90	T	DMT	Ph	+HTEA

For definition of abbreviations, see Table 1.

N^2 -Isobutyryl-2'-deoxyguanosine (1):

The acylation by transient protection method of R. A. Jones[44] was used. To a stirred mixture of 4.28 g (15.0 mmol) of 2'-deoxyguanosine monohydrate (that was first concentrated from dry pyridine) in 150 mL of dry pyridine that was cooled on an ice water bath was added 9.75 mL (76.8 mmol, 5.12 equiv) of chlorotrimethylsilane dropwise, over several minutes. After 30 min., 12.8 mL (76.9 mmol, 5.13 equiv.) of isobutyric anhydride was added dropwise, over several minutes. The ice bath was removed and stirring was continued for 2 h. The reaction mixture was then cooled on an ice water bath, and 30 mL of cold H_2O was added to the reaction. After 15 min., 30 mL of concentrated aqueous ammonia was added. The reaction was stirred for 30 min., and then concentrated. The residue was taken up in 100 mL of H_2O and extracted with Et_2O. The title compound was either crystallized from the aqueous layer, or was isolated by flash column chromatography.

N^2 -Isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (2):

The tritylation procedure of Jones[45] was modified such that no DMAP was used. To 3.37 g (10.0 mmol) of N^2 -isobutyryl-2'-deoxyguanosine (that was first concentrated from dry pyridine) in 50 mL of dry pyridine, was added 4.06 g (12.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The reaction was stirred for 15 h, and then concentrated. The residue was partitioned between CH_2Cl_2 and 0.5% aq. $NaHCO_3$, shaken, and separated. The organic layer was washed with 0.5% aq. $NaHCO_3$ and dried. The crude product was purified by flash chromatography.

N^4 -Benzoyl-2'-deoxycytidine (3):

This compound was prepared from 2'-deoxycytidine monohydrate by the same procedure used for the preparation of N^2 -isobutyryl-2'-deoxyguanosine except that 9.0 mL (77.5 mmol, 5.17 equiv.) of benzoyl chloride was used instead of isobutyric anhydride.

N^4 -Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (4):

This compound was prepared from N^4 -benzoyl-2'-deoxycytidine by the same procedure used for the preparation of N^2 -isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

N^6 -Benzoyl-2'-deoxyadenosine (5):

This compound was prepared from 2'-deoxyadenosine monohydrate by the same procedure used for the preparation of N^4 -benzoyl-2'-deoxycytidine.

N^6 -Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (6):

This compound was prepared from N^6 -benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of N^2 -isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

5'-O-(4,4'-Dimethoxytrityl)-thymidine (7):

This compound was prepared from thymidine by the same procedure used for the preparation of N^2 -isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilyl-N^2 -isobutyryl-2'-deoxyguanosine (8):

To a stirred solution of 2.00 g (3.13 mmol) of N^2 -isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine and 1.54 g (22.6 mmol, 7.22 equiv.) of imidazole in 12.5 mL of dry DMF was added 1.16 g (7.70 mmol, 2.46 equiv.) of t-butyldimethylsilyl chloride. The reaction was stirred at room temperature for 3.5 h and then concentrated. The residue was partitioned between $CH_2 Cl_2$ and $H_2 O$, shaken, and separated. The organics were washed with $H_2 O$ and concentrated (not dried). The crude residue was then stirred in 100 mL of 80% aq. HOAc for 1.5 h and then concentrated. The residue was partitioned between $CH_2 Cl_2$ and $H_2 O$, shaken, and separated. The organics were washed with sat. aq. $NaHCO_3$, $H_2 O$, and dried. The crude product was purified by flash chromatography on a 40 mm column using one column volume of 2% TEA in $CH_2 Cl_2$, then one column volume of 2% TEA and 2% MeOH in $CH_2 Cl_2$, and then 2% TEA and 4% MeOH in $CH_2 Cl_2$. The product was concentrated from toluene affording 1.18 g (83.7% yield).

3'-O-t-Butyldimethylsilyl-N^4 -benzoyl-2'-deoxycytidine (9):

This compound was prepared from N^4 -benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2 -isobutyryl-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilyl-N^6 -benzoyl-2'-deoxyadenosine (10):

This compound was prepared from N^6 -benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2 -isobutyryl-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilylthymidine (11):

This compound was prepared from 5'-O-(4,4'-dimethoxytrityl)-thymidine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2 -isobutyryl-2'-deoxyguanosine.

3'-O,N^3 -Dibenzylthymidine (12):

To a stirred solution of 2.18 g of 5'-O-(4,4'-dimethoxytrityl)-thymidine (4.00 mmol) in 52 mL of dry DMF was carefully added 2.00 g of a 60% oil dispersion of NaH. The reaction was stirred at room temperature for 5 min. To the mixture was added 4.77 mL (40.1 mmol, 10.0 equiv.) of benzyl bromide dropwise, over several minutes. After 1 h, the reaction was cooled

on an ice-water bath. Then, 12 mL of sat. aq. $NaHCO_3$ was carefully added (vigorous hydrogen gas evolution) dropwise, over several minutes. The mixture was stirred for 10 min, and then concentrated. The residue was then stirred in 100 mL of 80% aq. HOAc at room temperature for 1.5 h, and then concentrated. The crude residue was partitioned between CH_2 Cl_2 and H_2 O, shaken, and separated. The organic layer was washed with sat. aq. $NaHCO_3$, H_2 O, and then dried. The crude product was purified by flash chromatography on a 50 mm column using two column volumes of CH_2 Cl_2, two column volumes of 1% MeOH in CH_2 Cl_2, and then 2.5% MeOH in CH_2 Cl_2 as eluents. This afforded 1.49 g of product (88.2% yield) as a colorless solid.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2 -isosbutyrylguanine]-6'-phosphonate (13):

Literature methods[39] were adapted for the preparation of the title compound. To a solution of 106 mg of 3'-O-t-butyldimethylsilyl-N^2 -isobutyryl-2'-deoxyguanosine (0.236 mmol) and 294 mg of dicyclohexylcarbodiimide DCC (1.42 mmol, 6.02 equiv.) in 1.3 mL of dry DMSO was added 11.3 mg of methylphosphonic acid (0.118 mmol, 0.50 equiv.). The reaction was stirred at room temperature. After 18 h; dry pyridine (0.080 mL) and then 120 mg (0.236 mmol, 1.00 equiv.) of diphenyl [(triphenylphosphoranylidene)methyl]phosphonate[46] were added. Another 0.80 mL of dry DMSO was added. The reaction was stirred at room temperature. After 27 h, the reaction was diluted with CH_2 Cl_2, washed with 2.times.H_2 O, and dried. The crude material was flashed on a 25 mm column using one column volume of CH_2 Cl_2, then one column volume of 3% MeOH in CH_2 Cl_2, and then 6% MeOH in CH_2 Cl_2 as eluents. The product containing fractions were combined and concentrated. The product was purified again purified by flash chromatography on a 25 mm column using one column volume of 12.5% EtOAc in CH_2 Cl_2, then one column volume of 25% EtOAc in CH_2 Cl_2, and then 50% EtOAc in CH_2 Cl_2 as eluents. This procedure afforded 9.4 mg (6.0% yield) of product.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2 -isobutyrylguanine]-6'-phosphonate (14):

To a solution of 9.4 mg (0.0138 mmol) of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2

-isosbutyrylguanine]-6'-phosphonate in 20 mL of MeOH was added a catalytic amount of 10% Pd on carbon. The mixture was hydrogenated at 260 psi of H_2 (in a Parr reaction vessel) for 3 h. The mixture was filtered through Celite and concentrated. The crude product was purified by flash chromatography on a 15 mm column using one column volume of CH_2 Cl_2, then one column volume of 12.5% EtOAc in CH_2 Cl_2, then one column volume of 25% EtOAc in CH_2 Cl_2, and then 50% EtOAc in CH_2 Cl_2 as eluents. This procedure afforded 2.0 mg (21.3% yield) of product.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^4 -benzoylcytosine]-6'-phosphonate (15):

This compound is prepared from 3'-O-t-butyldimethylsilyl-N^4 -benzoyl-2'-deoxycytidine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2 -isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.4 -benzoylcytosine]-6'-phosphonate (16):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2 -isobutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^6 -benzoyladenine]-6'-phosphonate (17):

This compound is prepared from 3'-O-t-butyldimethylsilyl-N^6 -benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2 -isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.6 -benzoyladenine]-6'-phosphonate (18):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2 -isobutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-thymine]-6'-phosphonate (19):

This compound is prepared from 3'-O-t-butyldimethylsilylthymidine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2 -isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl), thymine]-6'-phosphonate (20):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2 -isobutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^3 -benzylthymine]-6'-phosphonate (21):

The title compound was prepared by modification of related known procedures.[13,19] To a stirred solution of 300 mg of 3'-O,N^3 -dibenzylthymidine (0.710 mmol) and 874 mg of. (4.24 mmol, 5.97 equiv.) of dicyclohexylcarbodiimide (DCC), in 2.37 mL of DMSO was added

0.356 mL of a 1.0M solution (0.356 mmol, 0.50 equiv.) of orthophosphoric acid (Aldrich) in DMSO. The reaction was stirred at room temperature. After 19 h, 0.237 mL of dry pyridine was added, followed by 412 mg (0.710 mmol, 1.0 equiv.) of diphenyl [(triphenylphosphoranylidene)methyl]phosphonate. The reaction was stirred for 31 h. The reaction mixture was partitioned between CH_2Cl_2 and H_2O, shaken and separated. The organic layer was washed with H_2O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 5% EtOAc in CH_2Cl_2, and then 10% EtOAc in CH_2Cl_2 as eluents. This afforded 334 mg (80.5% yield) of product.

Diphenyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (22):

To a stirred solution of 334 mg (0.513 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^3-benzylthymine]-6'-phosphonate in 7.7 mL of dry Et_2O was added 307 mg (1.03 mmol, 2.01 equiv.) of 2,4,6-tri-isopropylbenzenesulphonyl hydrazide,[47] followed by 0.143 mL of dry TEA. The reaction was refluxed for 14 h. The mixture was partitioned between Et_2O and sat. aq. $NaHCO_3$, shaken, and separated. The organic layer was washed with H_2O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 5% EtOAc in CH_2Cl_2, and then 10% EtOAc in CH_2Cl_2 as eluents. This afforded 244 mg (72.8% yield) of product.

Dimethyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (23):

Commercially available CsF (100 mg) was flame dried while under vacuum, and allowed to cool to room temperature. To the dried solid was added 3.00 mL of dry MeOH, followed by 143 mg (0.219 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate. The reaction was stirred for 20 h, and then concentrated. The residue was partitioned between CH_2Cl_2 and H_2O, shaken, and separated. The organics were washed with H_2O and dried. The residue was purified on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 2.5% MeOH in CH_2Cl_2, and then 5% MeOH in

CH_2Cl_2 as eluents. This procedure afforded 85.9 mg (74.0% yield) of product.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (24):
Known literature methods[48] were adapted to remove the benzyl protecting group from the 3'-oxygen. Dimethyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (3.0 mg, 0.00567 mmol) was added to a 4.4% solution of HCO_2H in MeOH (prepared from 96% HCO_2H) followed by a catalytic amount of 10% Pd on carbon. The reaction was stirred at room temperature for 19 h. The reaction was then filtered through Celite and concentrated. This procedure afforded 2.0 mg (80.6% yield) of product as a colorless solid.

Dibenzyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (25):
This procedure was based on a related procedure.[25] To a solution of 416 mg (0.638 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate in 3.0 mL of benzyl alcohol, was added 2.0 mL of a solution prepared by the addition of 200 mg of NaH to 16.7 mL of benzyl alcohol. After 1 h, the reaction mixture was diluted with 50 mL of Et_2O. Excess gaseous CO_2 was bubbled into the mixture. A gel like mixture formed which was dissolved in EtOAc. This solution was concentrated onto silica gel. The silica gel was loaded onto a previously equilibrated 25 mm column and eluted with one column volume of CH_2Cl_2, then one column volume of 10% EtOAc in CH_2Cl_2, and then 20% EtOAc in CH_2Cl_2 as eluents. This afforded 127 mg (29.3% yield) of product.

Diphenyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranoxyl)-N^2-isobutyrylguanine]-6'-phosphonate (26):
This reaction is based on a similar procedure by Barton et al.[30] To 5.00 mmol of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2-isobutyrylguanine]-6'-phosphonate in 100 mL of dry THF is added 5.5 mL (5.5 mmol, 1.1 equiv.) of a 1.00M solution of tetrabutylammonium fluoride (TBAF) in THF. The reaction is stirred at room temperature for 1 h. Then 20 mL of MeOH is added. The reaction is stirred

for 5 min., and then concentrated. The residue is purified by flash chromatography.

Diphenyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate (27):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate (28):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (29):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-th ymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate (30):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate and CsF in MeOH by the same procedure used for the preparation of dimethyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribo-hexofuranosyl)-N^3 -benzylthymine]-6'-phosphonate. After the aqueous extraction and drying, the crude product is purified by flash chromatography.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (31):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate (32):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (33):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid (34):

This reaction is based on a similar procedure by Barton et al.[30] To a stirred, ice-cooled mixture of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate in 150 mL of CH_2Cl_2 is added 1.98 mL (15.0 mmol, 3.0 equiv.) of bromotrimethylsilane dropwise, over several minutes. The reaction is stirred for 30 min., and then the ice bath is removed. After stirring for an additional 10 h, 20 mL of MeOH is added. The reaction is stirred for 5 min., and then concentrated. The product is used without further purification.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N_4 -benzoyl cytosine]-6'-phosphonic acid (35):

This compound is prepared from dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonic acid (36): This compound is prepared from dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonic acid (37): This compound was prepared from dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid (38): The entire crude [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid, from above, is heated in 150 mL of concentrated aqueous ammonia at 55° C. for 18 h, and then concentrated.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonic acid (39): This compound is prepared from [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-adenine]-6'-phosphonic acid (40): This compound is prepared from [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate (41): To 5.00 mmol of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate (that is first concentrated from dry pyridine) in 30 mL of dry pyridine, is added 2.03 g (6.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The

reaction is stirred for 15 h, and then concentrated. The residue is partitioned between CH_2Cl_2 and 0.5% aq. $NaHCO_3$, shaken, and separated. The organic layer is washed with 0.5% aq. $NaHCO_3$ and dried. The crude product is purified by flash chromatography.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate (42):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate (43):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (44):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl-N^2 -isobutyrylguanine]-6'-phosphonate.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate triethylammonium salt (45):

A mixture of 3.00 mmol of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate is stirred in 100 mL of concentrated aqueous ammonia

at room temperature. The reaction is monitored by TLC. After ca. 1 h, the mixture is concentrated. The product is purified by flash chromatography.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate triethylammonium salt (46):

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate triethylammonium salt (47):

This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt (48):

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate triethylammonium salt.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^2 -isobutyrylguanine (49):

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-guanine[49] by the same procedure used for the preparation of N^2 -isobutyryl-2'-deoxyguanosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^2-isobutyrylguanine (50):

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^2-isobutyrylguanine by the same procedure used for the preparation of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine (51):

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine[50] by the same procedure used for the preparation of N^4-benzoyl-2'-deoxycytidine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^4-benzoylcytosine (52):

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine by the same procedure used for the preparation of N^4-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine (53):

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine[49] by the same procedure used for the preparation of N^6-benzoyl-2'-deoxyadenosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^6-benzoyladenine (54):

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine by the same procedure used for the preparation of N^6-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-thymine (55):

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine[51] by the same procedure used for the preparation of 5'-O-(4,4'-dimethoxytrityl)-thymidine.

9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-Nxxx2.s up.2 -isobutyrylguanine (56):

This compound is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^2-isobutyrylguanine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine (57):

This compound is prepared from 1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^4-benzoylcytosine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^4-benzoyl-2'-deoxycytidine.

9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine (58):

This compound is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^6-benzoyladenine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^6-benzoyl-2'-deoxyadenosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thy mine (59):

This compound is prepared from 1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]- thymine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilylthymidine.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^2-isosbutyrylguanine]-6'-phosphonate (60):

This compound is prepared from 9-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N. sup.2 -isobutyrylguanine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^2-isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (61):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex- 5-enofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.2 -isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate (62):

This compound is prepared from 1-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N. sup.4 -benzoylcytosine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^4 -benzoylcytosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate (63):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex- 5-enofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl[1-(3-O+butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate (64):

This compound is prepared from 9-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N. sup.6 -benzoyladenine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-N^6 -benzoyladenine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (65):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex- 5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N. sup.6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate (66):

This compound is prepared from 1-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-th ymine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranos yl)-thymine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (67):This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-th ymine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (68):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (69):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (70):

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (71):

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (72):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (73):

This compound is prepared from diphenyl

[1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (74):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (75):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine], 6'-phosphonic acid (76):

This compound is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-15-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonic acid (77):

This compound is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of

[1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonic acid (78):
This compound is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonic acid (79):
This compound is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-guanine]-6'-phosphonic acid (80):
This compound is prepared from [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2 -isobutyrylguanine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-cytosine]-6'-phosphonic acid (81):
This compound is prepared from [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4 -benzoylcytosine]-6'-phosphonic acid by the same procedure used for the preparation of [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-adenine]-6'-phosphonic acid (82):
This compound is prepared from [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6 -benzoyladenine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-adenine]-6'-phosphonic acid.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (83):

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (84):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (85):This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (86):

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)- thymine]-6'-phosphonate.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt (87):

This compound is prepared from diphenyl

[9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate triethylammonium salt (88): This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate triethylammonium salt.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate triethylammonium salt (89): This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt (90): This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt.

Synthesis of Oligonucleotides

Oligonucleotides are synthesized from the 5'-end to the 3'-end. The phosphotriester method of oligonucleotide synthesis described by Sproat and Gait is used.[38] Appropriately protected 3'-O-(4,4'-dimethoxytrityl)-nucleosides having a free 5'-hydroxyl group are required for the solid phase synthesis.[52] These nucleosides are affixed to a long chain alkylamine controlled pore glass (LCAA/CPG) via a succinate linker using standard methods.[38] The 3'-O-DMT group on the support bound nucleoside is cleaved with 3% (v/v) dichloroacetic acid in 1,2-dichloroethane (DCE). After washing with DCE, and then pyridine, coupling of the appropriate monophenyl nucleoside-6'-phosphonate as its triethylammonium salt is effected with the coupling agent 1-mesitylenesulphonyl-3-nitro-1,2,4-triazole (MSNT) and 1-methylimidazole (NMI) in pyridine. This coupling is allowed to occur from 15-45 minutes. The support is then washed with pyridine. The oligo containing support is then treated with an Ac_2O/lutidine/DMAP is capping solution. The capping agent and its use is described by Atkinson and Smith.[53] After capping, the support is washed with first DCE, pyridine, and then DCE again. Then the cycle is repeated (*i.e.*, deprotection, coupling, capping). After the last coupling step, the fully protected oligonucleotide is cleaved from the support and fully deprotected using a mixture of pyridine-2-carbaldoxime and tetramethylguanidine in dioxane/water.[38] This deprotection is allowed to occur at 37° C. for 20 h. After drying in vacuo, the oligonucleotide is purified by either HPLC or polyacrylamide gel electrophoresis (PAGE).

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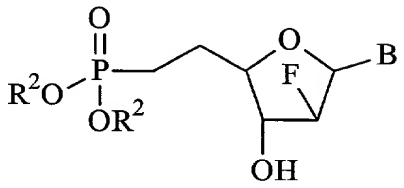
What is claimed is:

1. A compound having the formula:



wherein:

B is adenosine, N^6-benzoyladenine, thymine, guanine, or N^2-isobutyrylguanine; and

each R^2 is independently hydrogen, phenyl, alkyl (1-12C) or hydrogentriethylammonium ion.

2. The compound of claim 1 wherein B is guanine.

3. The compound of claim 2 wherein R^2 is hydrogen.

4. The compound of claim 3 wherein B is guanine.

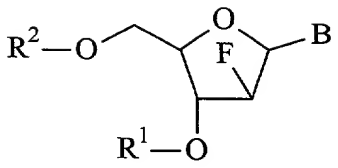
5. The compound of claim 3 wherein B is N^2-isobutyrylguanine.

6. The compound of claim 3 wherein B is adenine.

7. The compound of claim 3 wherein B is N^6-benzoyladenine.

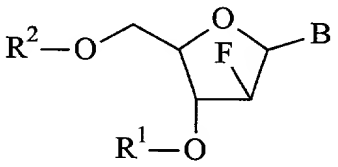
8. The compound of claim 3 wherein B is thymine.

9. The compound having the general formula:



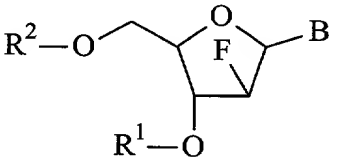
wherein B is N^2-isobutyrylguanine, R^1 and R^2 are both H.

10. The compound having the general formula:



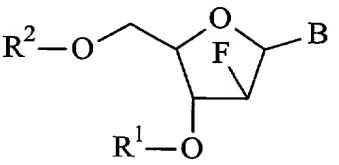
wherein B is N^4-benzoylcytosine, R^1 and R^2 are both H.

11. The compound having the general formula:



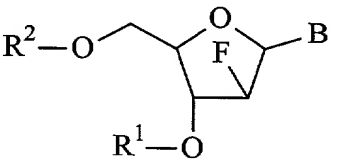
wherein B is N^6-benzoyladeneine, R^1 and R^2 are both H.

12. The compound having the general formula:



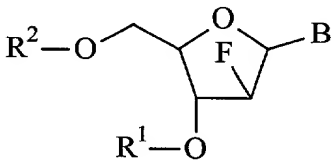
wherein B is N^2-isobutyrylguanine, R^1 is H and R^2 is 4,4'-dimethoxytrityl.

13. The compound having the general formula:



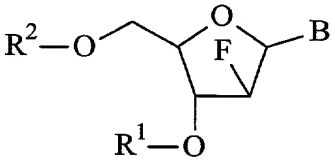
wherein B is N^4-benzoylcytosine, R^1 is H and R^2 is 4,4'-dimethoxytrityl.

14. The compound having the general formula:



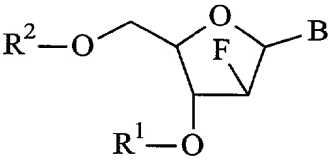
wherein B is N^6-benzoyladenine, R^1 is H and R^2 is 4,4'-dimethoxytrityl.

15. The compound having the general formula:



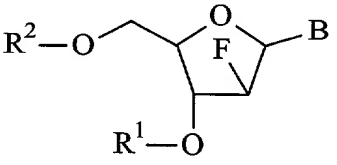
wherein B is thymine, R^1 is H and R^2 is 4,4'-dimethoxytrityl.

16. The compound having the general formula:



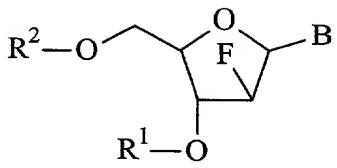
wherein B is N^2-isobutyrylguanine, R^1 is t-butyldimethylsilyl and R^2 is H.

17. The compound having the general formula:



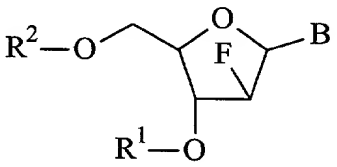
wherein B is N^4-benzoylcytosine, R^1 is t-butyldimethylsilyl and R^2 is H.

18. The compound having the general formula:



wherein B is N^6-benzoyladenine, R^1 is t-butyldimethylsilyl and R^2 is H.

19. The compound having the general formula:



wherein B is thymine, R^1 is t-butyldimethylsilyl and R^2 is H.

20. A modified oligonucleotide or derivative thereof comprising at least one nucleoside selected from the group consisting of a 2'-deoxy-2'-fluoro-ribonucleoside, an arabinonucleoside, a 2'-deoxy-arabinonucleoside; and a 2'-deoxy-2'-fluoro-arabinonucleoside.

21. The modified oligonucleotide of claim 20 wherein said nucleoside is a 2'-deoxy-arabinonucleoside.

22. The modified oligonucleotide of claim 20 wherein said nucleoside is a 2'-deoxy-2'-methylester-arabinonucleoside.

23. The modified oligonucleotide of claim 20 wherein said nucleoside is a 2'-deoxy-2'-fluoro-arabinonucleoside.

24. The modified oligonucleotide of claim 20 having a length of 2 to 30 nucleotides.

25. The modified oligonucleotide of claim 24 wherein at least one internucleoside linkage is selected from the group consisting of phosphorothioate, phosphorodithioate, morphilodate, piperazidate, methylphosphonate and phosphoroamidate.

26. The modified oligonucleotide of claim 20 wherein at least one internucleoside linkage is phosphorothioate.

Abstract

Novel oligonucleotides analogs and nucleoside analogs as well as methods for their synthesis are described. The oligonucleotides are useful in diagnostic and therapeutic applications. The oligonucleotides are stable to nuclease degradation.

NUCLEOSIDE 5'-METHYLENE PHOSPHONATES

FIELD OF THE INVENTION

This invention relates to novel methylene phosphonate nucleosides which exhibit antiviral and antitumor activity and novel oligonucleotides derived from methylene phosphonate nucleoside monomers that have enhanced nuclease stability. The invention also relates to processes for preparing the novel compounds, their derivatives and oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages. The oligonucleotides are resistant to nuclease degradation and are useful for diagnostic and therapeutic applications.

BACKGROUND ART

Antisense and triple helix oligonucleotides are synthetic oligonucleotides which bind complementary nucleic adds (i.e. sense strand RNA or duplex DNA sequences) via hydrogen bonding, thereby inhibiting expression of these sequences. Therapeutic intervention at the nucleic acid level using oligonucleotides offers a number of advantages. Inhibition of gene expression is more efficient than inhibition of the protein encoded by the gene since transcription of a single DNA sequence gives rise to multiple copies of mRNA which, in turn, are translated into many protein molecules.

Oligonucleotides have been used to inhibit gene expression in a variety of systems. There are several reviews that discuss this topic.[1-4] In addition, the use of oligonucleotides in sequence-specific recognition of double stranded DNA[5,6] as well as potential chemotherapeutic agents,[7] has been reviewed.

An important feature of the antisense oligomeric probes is the design of the backbone of the administered oligomer. Specifically, the backbone should contain internucleoside linkages that are stable in vitro and should be structured such that the oligomer is resistant to endogenous nucleases, such as nucleases that attack the phosphodiester linkage.[8] At the same time, the oligomer must also retain its ability to hybridize to the target DNA or RNA. In order to ensure these properties, a number of modified oligonucleotides have been constructed which contain alternate internucleoside linkages. Several of these oligonucleotides are described in Uhlmann, E. and Peyman, A., *Chemical Reviews* (1990) 90:543–584. Among these are methylphosphonates (wherein one of the phosphorous-linked oxygens has been replaced by methyl); phosphorothioates[8,9] (wherein sulphur replaces one of these oxygens) and various amidates (wherein NH_2 or an organic amine derivative, such as morpholidates or piperazidates, replace an oxygen). These substitutions confer enhanced stability, for the most part, but suffer from the drawback that they result in a chiral phosphorous in the linkage, thus leading to the formation of 2^n diastereomers where n is the number of modified diester linkages in the oligomer. The presence of these multiple diastereomers considerably weakens the capability of the modified oligonucleotide to hybridize to target sequences. Some of these substitutions also retain the ability to support a negative charge and the presence of charged groups decreases the ability of the compounds to penetrate cell membranes. There are numerous other disadvantages associated with these modified linkages, depending on the precise nature of the linkage. Phosphorodithioate modified backbones have been made.[9,10] These modified oligonucleotides are nuclease resistant and are diastereomerically pure. However, these modifications further reduce the affinity of the oligonucleotide for its intended target.[10c] A variety of modified nonionic[11] oligonucleotides including methylphosphonate, phosphoroamidate, and phosphotriesters generally are either composed of a mixture of diastereomers, have a low affinity for intended targets, or both.

A deoxyoligonucleotide comprised from nucleotide monomers that contain a methylene (—CH_2—) group substituted for the 5'-oxygen may be resistant to nucleases, especially those that leave a 3'-phosphate moiety after cleavage of the internucleotidic bond. This results from the fact that the requisite P—C bond can not be cleaved under normal physiological conditions. Additionally, a single diastereomerically pure deoxyoligonucleotide could be prepared, as the internucleotide phosphorous linkages would be achiral. We refer to the nucleotides containing a methylene (—CH_2—) group substituted for the 5'-oxygen as 5'-methylene phosphonates.

The preparation of ribo (ie 2'-OH) 5'-methylene phosphonates is well documented in the literature.[12] Uridine,[13-15] adenosine,[13-15] and guanosine[16] 5'-methylene phosphonates have been prepared. A number of analogues of adenosine 5'-methylene phosphonate have been prepared.[17-23] In addition, ribavirin 5'-methylene phosphonate,[24] as well as a ribo 5'-methylene phosphonate containing thiazole-4-carboxamide as the base, has been prepared.[25] Ribo compounds having a 3'-methylene phosphonate have also been prepared.[26-28]

There are very few reports of 2'-deoxy 5'-methylene phosphonates in the literature, and these are all related to thymidine. Only the syntheses of 5'-methylene phosphonates of thymidine,[29] 3'-azidothymidine (AZT),[30,31] and 2'-deoxy-5-fluoro-uridine[32] have been reported. There have been no reports on the syntheses of 5'-methylene phosphonates derived from 2'-deoxyadenosine, 2'-deoxycytidine, or 2'-deoxyguanosine. There also have been no reports on the synthesis of 5' methylene phosphonate nucleosides having 5-iodouracil, 2-aminopurine or 2,6-diaminopurine as the base. The 5-iodouridine 5' methylene phosphonate compound would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from thymidine as described for compounds 33 and 37 below. The 2-aminopurine and 2,6-diaminopurine nucleoside 5' methylene phosphonates would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from deoxyadenosine as described for compounds 36 and 40 below.

Several ribo 5'-methylene phosphonate dimers have been synthesized. These include $UpCH_2U$ and $UpCH_2A$.[33,34] Several ribo 3'-methylene phosphonate dimers,[33] as well as a trimer[28] have been synthesized. These ribo dimers and trimer were prepared using the diester method of oligonucleotide synthesis.[35,36] This method suffers from low product yields, and difficulties in purification of the final product.[35,36] The method is generally not useful in the preparation of longer oligonucleotides. Recently, a ribo oligonucleotide 10-mer consisting of 5'-methylene phosphonates, $ApA(pCH_2A)_8$, was prepared enzymatically using polynucleotide phosphorylase.[37] This technique, however, cannot be used for the preparation of oligonucleotides having a defined sequence of mixed bases.

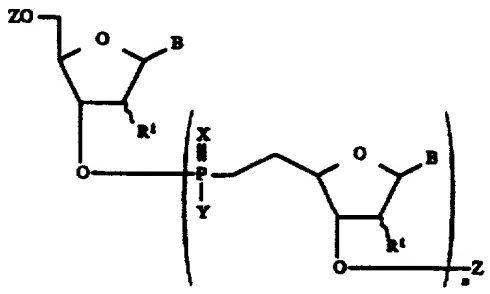
Only one 2'-deoxy dimer, $TpCH_2T$, and one 2'-deoxy trimer, $TpCH_2TpCH_2T$, have been reported in the literature.[29] Only the 5'-methylene phosphonate derived from thymidine was used in the dimer and trimer. No mixed base 2'-deoxy 5'-methylene phosphonate dimers or longer mixed

base. 2'-deoxy 5'-methylene phosphonate oligonucleotides have been reported. Additionally, no 2'-deoxy 5'-methylene phosphonate oligonucleotides longer than a 3-mer of any kind have been reported. However, recently the synthesis of oligodeoxynucleotides containing 5'-methylene phosphonates of 2'-deoxy-4'-carbocyclic nucleosides has been reported W. Frick and S. W. Schneller, Meetings Abstract, Conference on Nucleic Acid Therapeutics, Jan. 13–17, 1991, Clearwater Beach, Fla., p63).

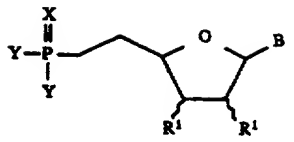
The present invention relates to the synthesis of 2'-deoxy-5'-methylene phosphonate oligonucleotides of length 2–30 of mixed base composition. These oligodeoxynucleotides are prepared using the phosphotriester method[36] from suitably protected 2'-deoxy 5'-methylene phosphonate nucleotide monomers. We prepared novel 5'-methylene phosphonates in both a protected form that was suitable for oligonucleotide synthesis, as well as in a completely deprotected form. Some of the novel 5'-methylene phosphonates that were prepared were derived from 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyguanosine. The monomers described herein are suitable for solid phase oligonucleotide synthesis by triester chemistry. Previous methods utilized diester chemistry which is more difficult and generates low yields of product. Oligonucleotides containing 2'-deoxy-2'-fluoro-ribonucleotides are of interest because the conformation of the sugar closely resembles that of RNA and consequently these oligonucleotides have a higher affinity to DNA than normal oligodeoxyribonucleotides (M. Ikehara, Heterocycles 1984, 21, 75).

DISCLOSURE OF THE INVENTION

The present invention discloses oligonucleotides and methods for their synthesis of formula (I):



and stereoisomers thereof, wherein each B is independently a purine or pyrimidine base or modified form each Z is independently a noninterfering substituent, preferably hydrogen, PO_3^- or a protecting group; each R^1 is independently hydrogen, hydroxyl, fluorine or methyl ester; each Y is independently OR^2, $N(R^2)_2$ or SR^2 wherein, each R^2 is independently hydrogen or alkyl (1–12 C); X is selected from oxygen and sulfur; n is an integer from 1 to 200. Bases such as adenine, guanine, cytosine, thymine and uracil as well as modified forms (base analogs) such as 5-methylcytosine, aziridinylcytosine, 8-hydroxy-N^6-methyladenine, pseudoisocytosine and inosine are preferred. The oligonucleotides contain one or more 5' methylene phosphonate linkages. The oligonucleotides may be synthesized from derivatives disclosed herein of monomers of formula (II):



wherein B is a purine or pyrimidine base or modified form; each R^1 is independently hydrogen, hydroxyl, fluorine or methyl ester; each Y is independently OR^2, $N(R^2)_2$ or SR^2 wherein, each R^2 is independently hydrogen or alkyl (1–12 C); and X is selected from oxygen and sulfur. Bases such as guanine, adenine, cytosine, thymine, uracil, iodouracil, 8-hydroxy-N^6-methyladenine, aziridinylcytosine, 2-aminopurine, 2, 6-diaminopurine or other base analogs or altered forms may be utilized. Alternative monomer structures, such as 2',3' epoxides and 2',3'dideoxy didehydro sugars may also be synthesized.

The free 5'-methylene phosphonate nucleosides present enzymatically nonhydrolysable isosteres of mononucleotides. As such they can be converted intracellularly by cellular kinases to the corresponding nucleoside phosphono triphosphates, incorporated into DNA by polymerases and thus interfere with cellular metabolism. Thus, such nucleoside phosphonates potentially exhibit antiviral and antitumour activity. For example, several acyclic methylene phosphonates such as the methylene phosphonates derived from ganciclovir, and acyclovir are potent antivirals.[39-42] Other nucleoside phosphonates have been claimed in a patent application (Elmer Reist et al, Stanford Research Institute, PCT publication no. WO 84/04748). The novel 5'-methylene compounds that are described herein thus have useful antiviral or antitumour activities.

The oligonucleotide and nucleoside monomer compounds possess antiviral activity and can be used in the control or prevention of viral infections, e.g. of herpes simplex vital infections. The in vitro activity of the compounds of formula I and their tautomers in inhibiting herpes simplex virus type 2 (HSV-2) can be demonstrated by means of the following plaque reduction procedure. Host VERO cells are infected with virus stock containing a known number of infectious virions in the presence of various concentrations of compound. Plaques in the cell monolayer are then counted and compared to untreated controls and to acydovir treated controls. The degree of inhibition at each concentration of compound is expressed as a percentage of the control titer (100%). The IC_{50} value, namely the concentration of compound which inhibits viral activity by 50%, is then calculated. The results that are obtained with representative compounds show that virus titer reductions occur.

The compounds disclosed herein can be used as medicaments in the form of pharmaceutical preparations which contain them in association with a compatible pharmaceutical carrier material. This can be an organic or inorganic carrier suitable for enteral, e.g. oral, or parenteral administration. Examples of such carriers are water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkylene glycols and petroleum jelly. The pharmaceutical preparations can be made up in a solid form, e.g. as tablets, dragees, suppositories or capsules, or in a liquid form, e.g. as solutions, suspensions or emulsions; they may be subjected to standard pharmaceutical operations, e.g. sterilization and/or may contain adjuvants, e.g. preserving, stabilizing, wetting or emulsifying agents, salts for varying the osmotic pressure or buffers. The compounds may also be formulated in a manner suitable for administration as an aerosol. They may also contain other therapeutically valuable substances.

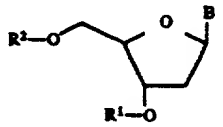
The compounds disclosed herein and their tautomers can be administered for the control or prevention of viral infection, such as herpes simplex viral infections, to warm-blooded animals in need of such treatment. The disclosed compounds and their tautomers can be administered to adult humans in a daily dosage of from about 1 to 1000 mg, preferably about 5 to 500 mg. The daily dosage may be administered as a single dose or in divided doses. The above dosage range is given by way of example only and can be varied upwards or downwards depending on factors such as the particular compound being administered, the route of administration, the severity of the indication being treated and the condition of the patient.

Experimental Section

General. Flash chromatography refers to the procedure of Still et. al.[43] Drying refers to drying over Na_2SO_4, filtration, and concentration. All reactions requiring dry solvents were run under a dry argon atmosphere.

The following six tables show structures for compounds 1 through 90.

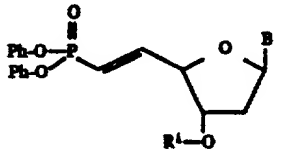
TABLE 1



Compound	B	R^1	R^2
1	G^{ib}	H	H
3	C^{Bz}	H	H
5	A^{Bz}	H	H
2	G^{ib}	H	DMT
4	C^{Bz}	H	DMT
6	A^{Bz}	H	DMT
7	T	H	DMT
8	G^{ib}	TBS	H
9	C^{Bz}	TBS	H
10	A^{Bz}	TBS	H
11	T	TBS	H
12	T^{Bz}	Bz	H

For tables 1–6; G = guanine; C = cytosine; A = adenine; T = thymine; G^{ib} = N^2-isobutyrylguanine; C^{Bz} = N^4-benzoylcytosine; A^{Bz} = N^6-benzoyladenine; T^{Bz} = N^3-benzylthymine; Bz = benzyl; DMT = 4,4'-dimethoxytrityl; TBS = t-butyldimethylsilyl; +HTEA = hydrogentriethylammonium

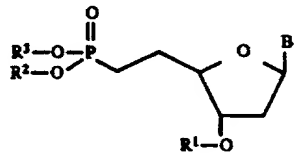
TABLE 2



Compound	B	R^1
13	G^{ib}	TBS
15	C^{Bz}	TBS
17	A^{Bz}	TBS
19	T	TBS
21	T^{Bz}	Bz

For definition of abbreviations, see Table 1.

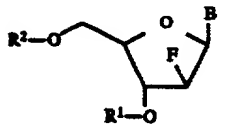
TABLE 3



Compound	B	R^1	R^2	R^3
14	G^{ib}	TBS	Ph	Ph
16	C^{Bz}	TBS	Ph	Ph
18	A^{Bz}	TBS	Ph	Ph
20	T	TBS	Ph	Ph
22	T^{Bz}	Bz	Ph	Ph
23	T^{Bz}	Bz	Me	Me
24	T^{Bz}	H	Me	Me
25	T^{Bz}	Bz	Bz	Bz
26	G^{ib}	H	Ph	Ph
27	C^{Bz}	H	Ph	Ph
28	A^{Bz}	H	Ph	Ph
29	T	H	Ph	Ph
30	G^{ib}	H	Me	Me
31	C^{Bz}	H	Me	Me
32	A^{Bz}	H	Me	Me
33	T	H	Me	Me
34	G^{ib}	H	H	H
35	C^{Bz}	H	H	H
36	A^{Bz}	H	H	H
37	T	H	H	H
38	G	H	H	H
39	C	H	H	H
40	A	H	H	H
41	G^{ib}	DMT	Ph	Ph
42	C^{Bz}	DMT	Ph	Ph
43	A^{Bz}	DMT	Ph	Ph
44	T	DMT	Ph	Ph
45	G^{ib}	DMT	Ph	+HTEA
46	C^{Bz}	DMT	Ph	+HTEA
47	A^{Bz}	DMT	Ph	+HTEA
48	T	DMT	Ph	+HTEA

For definition of abbreviations, see Table 1.

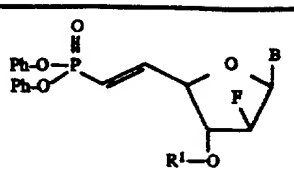
TABLE 4



Compound	B	R^1	R^2
49	G^{ib}	H	H
51	C^{Bz}	H	H
53	A^{Bz}	H	H
50	G^{ib}	H	DMT
52	C^{Bz}	H	DMT
54	A^{Bz}	H	DMT
55	T	H	DMT
56	G^{ib}	TBS	H
57	C^{Bz}	TBS	H
58	A^{Bz}	TBS	H
59	T	TBS	H

For definition of abbreviations, see Table 1.

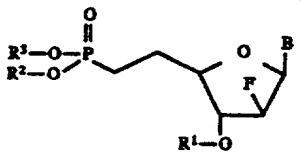
TABLE 5



Compound	B	R^1
60	G^{ib}	TBS
62	C^{Bz}	TBS
64	A^{Bz}	TBS
66	T	TBS

For definition of abbreviations, see Table 1.

TABLE 6



Compound	B	R^1	R^2	R^3
61	G^{ib}	TBS	Ph	Ph
63	C^{Bz}	TBS	Ph	Ph
65	A^{Bz}	TBS	Ph	Ph
67	T	TBS	Ph	Ph
68	G^{ib}	H	Ph	Ph
69	C^{Bz}	H	Ph	Ph
70	A^{Bz}	H	Ph	Ph
71	T	H	Ph	Ph
72	G^{ib}	H	Me	Me
73	C^{Bz}	H	Me	Me
74	A^{Bz}	H	Me	Me
75	T	H	Me	Me
76	G^{ib}	H	H	H
77	C^{Bz}	H	H	H
78	A^{Bz}	H	H	H
79	T	H	H	H
80	G	H	H	H
81	C	H	H	H
82	A	H	H	H
83	G^{ib}	DMT	Ph	Ph
84	C^{Bz}	DMT	Ph	Ph
85	A^{Bz}	DMT	Ph	Ph
86	T	DMT	Ph	Ph
87	G^{ib}	DMT	Ph	+HTEA
88	C^{Bz}	DMT	Ph	+HTEA
89	A^{Bz}	DMT	Ph	+HTEA
90	T	DMT	Ph	+HTEA

For definition of abbreviations, see Table 1.

N^2-Isobutyryl-2'-deoxyguanosine (1)

The acylation by transient protection method of R. A. Jones[44] was used. To a stirred mixture of 4.28 g (15.0 mmol) of 2'-deoxyguanosine monohydrate (that was first concentrated from dry pyridine) in 150 mL of dry pyridine that was cooled on an ice water bath was added 9.75 mL (76.8 mmol, 5.12 equiv) of chlorotrimethylsilane dropwise, over several minutes. After 30 min., 12.8 mL (76.9 mmol, 5.13 equiv.) of isobutyric anhydride was added dropwise, over several minutes. The ice bath was removed and stirring was continued for 2 h. The reaction mixture was then cooled on an ice water bath, and 30 mL of cold H_2O was added to the reaction. After 15 min., 30 mL of concentrated aqueous ammonia was added. The reaction was stirred for 30 min., and then concentrated. The residue was taken up in 100 mL of H_2O and extracted with Et_2O. The title compound was either crystallized from the aqueous layer, or was isolated by flash column chromatography.

N^2-Isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (2)

The tritylation procedure of Jones[45] was modified such that no DMAP was used. To 3.37 g (10.0 mmol) of N^2-isobutyryl-2'-deoxyguanosine (that was first concentrated from dry pyridine) in 50 mL of dry pyridine, was added 4.06 g (12.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The reaction was stirred for 15 h, and then concentrated. The residue was partitioned between CH_2Cl_2 and 0.5% aq. $NaHCO_3$, shaken, and separated. The organic layer was washed with 0.5% aq. $NaHCO_3$ and dried. The crude product was purified by flash chromatography.

N^4-Benzoyl-2'-deoxycytidine (3)

This compound was prepared from 2'-deoxycytidine monohydrate by the same procedure used for the preparation of N^2-isobutyryl-2'-deoxyguanosine except that 9.0 mL (77.5 mmol, 5.17 equiv.) of benzoyl chloride was used instead of isobutyric anhydride.

N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (4)

This compound was prepared from N^4-benzoyl-2'-deoxycytidine by the same procedure used for the preparation of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

N^6-Benzoyl-2'-deoxyadenosine (5)

This compound was prepared from 2'-deoxyadenosine monohydrate by the same procedure used for the preparation of N^4-benzoyl-2'-deoxycytidine.

N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (6)

This compound was prepared from N^6-benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

5'-O-(4,4'-Dimethoxytrityl)-thymidine (7)

This compound was prepared from thymidine by the same procedure used for the preparation of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine (8)

To a stirred solution of 2.00 g (3.13 mmol) of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine and 1.54 g (22.6 mmol, 7.22 equiv.) of imidazole in 12.5 mL of dry DMF was added 1.16 g (7.70 mmol, 2.46 equiv.) of t-butyldimethylsilyl chloride. The reaction was stirred at room temperature for 3.5 h and then concentrated. The residue was partitioned between CH_2Cl_2 and H_2O, shaken, and separated. The organics were washed with H_2O and concentrated (not dried). The crude residue was then stirred in 100 mL of 80% aq. HOAc for 1.5 h and then concentrated. The residue was partitioned between CH_2Cl_2 and H_2O, shaken, and separated. The organics were washed with sat. aq. $NaHCO_3$, H_2O, and dried. The crude product was purified by flash chromatography on a 40 mm column using one column volume of 2% TEA in CH_2Cl_2, then one column volume of 2% TEA and 2% MeOH in CH_2Cl_2, and then 2% TEA and 4% MeOH in CH_2Cl_2. The product was concentrated from toluene affording 1.18 g (83.7% yield).

3'-O-t-Butyldimethylsilyl-N^4-benzoyl-2'-deoxycytidine (9)

This compound was prepared from N^4-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilyl-N^6-benzoyl-2'-deoxyadenosine (10)

This compound was prepared from N^6-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilylthymidine (11)

This compound was prepared from 5'-O-(4,4'-dimethoxytrityl)-thymidine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine.

3'-O,N^3-Dibenzylthymidine (12)

To a stirred solution of 2.18 g of 5'-O-(4,4'-dimethoxytrityl)-thymidine (4.00 mmol) in 52 mL of dry DMF was carefully added 2.00 g of a 60% oil dispersion of NaH. The reaction was stirred at room temperature for 5 min. To the mixture was added 4.77 mL (40.1 mmol, 10.0 equiv.) of benzyl bromide dropwise, over several minutes. After 1 h, the reaction was cooled on an ice-water bath. Then, 12 mL of sat. aq. $NaHCO_3$ was carefully added (vigorous hydrogen gas evolution) dropwise, over several minutes. The mixture was stirred for 10 min, and then concentrated. The residue was then stirred in 100 mL of 80% aq. HOAc at room temperature for 1.5 h, and then concentrated. The crude residue was partitioned between CH_2Cl_2 and H_2O, shaken, and separated. The organic layer was washed with sat. aq. $NaHCO_3$, H_2O, and then dried. The crude product was purified by flash chromatography on a 50 mm column using two column volumes of CH_2Cl_2, two column volumes of 1% MeOH in CH_2Cl_2, and then 2.5% MeOH in CH_2Cl_2 as eluents. This afforded 1.49 g of product (88.2% yield) as a colorless solid.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanine]-6'-phosphonate (13)

Literature methods[39] were adapted for the preparation of the title compound. To a solution of 106 mg of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine (0.236 mmol) and 294 mg of dicyclohexylcarbodiimide DCC (1.42 mmol, 6.02 equiv.) in 1.3 mL of dry DMSO was added 11.3 mg of methylphosphonic acid (0.118 mmol, 0.50 equiv.). The reaction was stirred at room temperature. After 18 h; dry pyridine (0.080 mL) and then 120 mg (0.236 mmol, 1.00 equiv.) of diphenyl [(triphenylphosphoranylideae)methyl] phosphonate[46] were added. Another 0.80 mL of dry DMSO was added. The reaction was stirred at room temperature. After 27 h, the reaction was diluted with CH_2Cl_2, washed with 2×H_2O, and dried. The crude material was flashed on a 25 mm column using one column volume of CH_2Cl_2, then one column volume of 3% MeOH in CH_2Cl_2, and then 6% MeOH in CH_2Cl_2 as eluents. The product containing fractions were combined and concentrated. The product was purified again purified by flash chromatography on a 25 mm column using one column volume of 12.5% EtOAc in CH_2Cl_2, then one column volume of 25% EtOAc in CH_2Cl_2, and then 50% EtOAc in CH_2Cl_2 as eluents. This procedure afforded 9.4 mg (6.0% yield) of product.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (14)

To a solution of 9.4 mg (0.0138 mmol) of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanine]-6'-phosphonate in 20 mL of MeOH was added a catalytic amount of 10% Pd on carbon. The mixture was hydrogenated at 260 psi of H_2 (in a Parr reaction vessel) for 3 h. The mixture was filtered through Celite and concentrated. The crude product was purified by flash chromatography on a 15 mm column using one column volume of CH_2Cl_2, then one column volume of 12.5% EtOAc in CH_2Cl_2, then one column volume of 25% EtOAc in CH_2Cl_2, and then 50% EtOAc in CH_2Cl_2 as eluents. This procedure afforded 2.0 mg (21.3% yield) of product.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (15)

This compound is prepared from 3'-O-t-butyldimethylsilyl-N^4-benzoyl-2'-deoxycytidine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (16)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (17)

This compound is prepared from 3'-O-t-butyldimethylsilyl-N^6-benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (18)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate (19)

This compound is prepared from 3'-O-t-butyldimethylsilylthymidine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl), thymine]-6'-phosphonate (20)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanosine]-6'-phosphonate.

Diphenyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^3-benzylthymine]-6'-phosphonate (21)

The title compound was prepared by modification of related known procedures.[13,19] To a stirred solution of 300 mg of 3'-O,N^3-dibenzylthymidine (0.710 mmol) and 874 mg of. (4.24 mmol, 5.97 equiv.) of dicyclohexylcarbodiimide (DCC), in 2.37 mL of DMSO was added 0.356 mL of a 1.0M solution (0.356 mmol, 0.50 equiv.) of orthophosphoric acid (Aldrich) in DMSO. The reaction was stirred at room temperature. After 19 h, 0.237 mL of dry pyridine was

added, followed by 412 mg (0.710 mmol, 1.0 equiv.) of diphenyl [(triphenylphosphoranylidene)methyl] phosphonate. The reaction was stirred for 31 h. The reaction mixture was partitioned between CH_2Cl_2 and H_2O, shaken and separated. The organic layer was washed with H_2O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 5% EtOAc in CH_2Cl_2, and then 10% EtOAc in CH_2Cl_2 as eluents. This afforded 334 mg (80.5% yield) of product.

Diphenyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (22)

To a stirred solution of 334 mg (0.513 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^3-benzylthymine]-6'-phosphonate in 7.7 mL of dry Et_2O was added 307 mg (1.03 mmol, 2.01 equiv.) of 2,4,6-tri-isopropylbenzenesulphonyl hydrazide,[47] followed by 0.143 mL of dry TEA. The reaction was refluxed for 14 h. The mixture was partitioned between Et_2O and sat. aq. $NaHCO_3$, shaken, and separated. The organic layer was washed with H_2O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 5% EtOAc in CH_2Cl_2, and then 10% EtOAc in CH_2Cl_2 as eluents. This afforded 244 mg (72.8% yield) of product.

Dimethyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (23)

Commercially available CsF (100 mg) was flame dried while under vacuum, and allowed to cool to room temperature. To the dried solid was added 3.00 mL of dry MeOH, followed by 143 mg (0.219 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate. The reaction was stirred for 20 h, and then concentrated. The residue was partitioned between CH_2Cl_2 and H_2O, shaken, and separated. The organics were washed with H_2O and dried. The residue was purified on a 25 mm column using one column volume of CH_2Cl_2, one column volume of 2.5% MeOH in CH_2Cl_2, and then 5% MeOH in CH_2Cl_2 as eluents. This procedure afforded 85.9 mg (74.0% yield) of product.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (24)

Known literature methods[48] were adapted to remove the benzyl protecting group from the 3'-oxygen. Dimethyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (3.0 mg, 0.00567 mmol) was added to a 4.4% solution of HCO_2H in MeOH (prepared from 96% HCO_2H) followed by a catalytic amount of 10% Pd on carbon. The reaction was stirred at room temperature for 19 h. The reaction was then filtered through Celite and concentrated. This procedure afforded 2.0 mg (80.6% yield) of product as a colorless solid.

Dibenzyl [1-(3-O-Benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate (25)

This procedure was based on a related procedure.[25] To a solution of 416 mg (0.638 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^3-benzylthymine]-6'-phosphonate in 3.0 mL of benzyl alcohol, was added 2.0 mL of a solution prepared by the addition of 200 mg of NaH to 16.7 mL of benzyl alcohol. After 1 h, the reaction mixture was diluted with 50 mL of Et_2O. Excess gaseous CO_2 was bubbled into the mixture. A gel like mixture formed which was dissolved in EtOAc. This solution was concentrated onto silica gel. The silica gel was loaded onto a previously equilibrated 25 mm column and eluted with one column volume of CH_2Cl_2, then one column volume of 10% EtOAc in CH_2Cl_2, and then 20% EtOAc in CH_2Cl_2 as eluents. This afforded 127 mg (29.3% yield) of product.

Diphenyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranoxyl)-N^2-isobutyrylguanine]-6'-phosphonate (26)

This reaction is based on a similar procedure by Barton et al.[30] To 5.00 mmol of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate in 100 mL of dry THF is added 5.5 mL (5.5 mmol, 1.1 equiv.) of a 1.00M solution of tetrabutylammonium fluoride (TBAF) in THF. The reaction is stirred at room temperature for 1 h. Then 20 mL of MeOH is added. The reaction is stirred for 5 min., and then concentrated. The residue is purified by flash chromatography.

Diphenyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (27)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (28)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (29)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (30)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate and CsF in MeOH by the same procedure used for the preparation of dimethyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribo-hexofuranosyl)-N^3-benzylthymine]-6'-phosphonate. After the aqueous extraction and drying, the crude product is purified by flash chromatography.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (31)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (32)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (33)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid (34)

This reaction is based on a similar procedure by Barton et al.[30] To a stirred, ice-cooled mixture of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate in 150 mL of CH_2Cl_2 is added 1.98 mL (15.0 mmol, 3.0 equiv.) of bromotrimethylsilane dropwise, over several minutes. The reaction is stirred for 30 min., and then the ice bath is removed. After stirring for an additional 10 h, 20 mL of MeOH is added. The reaction is stirred for 5 min., and then concentrated. The product is used without further purification.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N_4-benzoyl cytosine]-6'-phosphonic acid (35)

This compound is prepared from dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonic acid (36)

This compound is prepared from dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonic acid (37)

This compound was prepared from dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid (38)

The entire crude [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid, from above, is heated in 150 mL of concentrated aqueous ammonia at 55° C. for 18 h, and then concentrated.

[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonic acid (39)

This compound is prepared from [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-adenine]-6'-phosphonic acid (40)

This compound is prepared from [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (41)

To 5.00 mmol of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (that is first concentrated from dry pyridine) in 30 mL of dry pyridine, is added 2.03 g (6.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The reaction is stirred for 15 h, and then concentrated. The residue is partitioned between CH_2Cl_2 and 0.5% aq. $NaHCO_3$, shaken, and separated. The organic layer is washed with 0.5% aq. $NaHCO_3$ and dried. The crude product is purified by flash chromatography.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (42)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (43)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate (44)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl-N^2-isobutyrylguanine]-6'-phosphonate.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt (45)

A mixture of 3.00 mmol of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate is stirred in 100 mL of concentrated aqueous ammonia at room temperature. The reaction is monitored by TLC. After ca. 1 h, the mixture is concentrated. The product is purified by flash chromatography.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate triethylammonium salt (46)

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate triethylammonium salt (47)

This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt (48)

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^2-isobutyrylguanine (49)

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-guanine[49] by the same procedure used for the preparation of N^2-isobutyryl-2'-deoxyguanosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^2-isobutyrylguanine (50)

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^2-isobutyrylguanine by the same procedure used for the preparation of N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine (51)

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine[50] by the same procedure used for the preparation of N^4-benzoyl-2'-deoxycytidine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^4-benzoylcytosine (52)

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine by the same procedure used for the preparation of N^4-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine (53)

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine[49] by the same procedure used for the preparation of N^6-benzoyl-2'-deoxyadenosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^6-benzoyladenine (54)

This compound is prepared from 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine by the same procedure used for the preparation of N^6-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-thymine (55)

This compound is prepared from 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine[51] by the same procedure used for the preparation of 5'-O-(4,4'-dimethoxytrityl)-thymidine.

9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^2-isobutyrylguanine (56)

This compound is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^2-isobutyrylguanine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^2-isobutyryl-2'-deoxyguanosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-benzoylcytosine (57)

This compound is prepared from 1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)- 2-fluoro-β-D-arabinofuranosyl]-N^4-benzoylcytosine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^4-benzoyl-2'-deoxycytidine.

9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^6-benzoyladenine (58)

This compound is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-N^6-benzoyladenine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilyl-N^6-benzoyl-2'-deoxyadenosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine (59)

This compound is prepared from 1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-thymine by the same procedure used for the preparation of 3'-O-t-butyldimethylsilylthymidine.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^2-isosbutyrylguanine]-6'-phosphonate (60)

This compound is prepared from 9-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) -N^2-isobutyrylguanine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^2-isosbutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (61)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (62)

This compound is prepared from 1-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) -N^4-benzoylcytosine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (63)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O+butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (64)

This compound is prepared from 9-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) -N^6-benzoyladenine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (65)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate (66)

This compound is prepared from 1-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) -thymine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (67)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (68)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (69)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (70)

This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (71)

This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (72)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (73)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Dimethyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (74)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Dimethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (75)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine], 6'-phosphonic acid (76)

This compound is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-15 -D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonic acid (77)

This compound is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonic acid (78)

This compound is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonic acid (79)

This compound is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-guanine]-6'-phosphonic acid (80)

This compound is prepared from [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-cytosine]-6'-phosphonic acid (81)

This compound is prepared from [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonic acid by the same procedure used for the preparation of [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-adenine]-6'-phosphonic acid (82)

This compound is prepared from [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-adenine]-6'-phosphonic acid.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate (83)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate (84)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure

used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate (85)

This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (86)

This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt (87)

This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^2-isobutyrylguanine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate triethylammonium salt (88)

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^4-benzoylcytosine]-6'-phosphonate triethylammonium salt.

Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate triethylammonium salt (89)

This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N^6-benzoyladenine]-6'-phosphonate triethylammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt (90)

This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate triethylammonium salt.

Synthesis of Oligonucleotides

Oligonucleotides are synthesized from the 5'-end to the 3'-end. The phosphotriester method of oligonucleotide synthesis described by Sproat and Gait is used.[36] Appropriately protected 3'-O-(4,4'-dimethoxytrityl)-nucleosides having a free 5'-hydroxyl group are required for the solid phase synthesis.[52] These nucleosides are affixed to a long chain alkylamine controlled pore glass (LCAA/CPG) via a succinate linker using standard methods.[36] The 3'-O-DMT group on the support bound nucleoside is cleaved with 3% (v/v) dichloroacetic acid in 1,2-dichloroethane (DCE). After washing with DCE, and then pyridine, coupling of the appropriate monophenyl nucleoside-6'-phosphonate as its triethylammonium salt is effected with the coupling agent 1-mesitylenesulphonyl-3-nitro-1,2,4-triazole (MSNT) and 1-methylimidazole (NMI) in pyridine. This coupling is allowed to occur from 15–45 minutes. The support is then washed with pyridine. The oligo containing support is then treated with an Ac_2O/lutidine/DMAP is capping solution. The capping agent and its use is described by Atkinson and Smith.[53] After capping, the support is washed with first DCE, pyridine, and then DCE again. Then the cycle is repeated (ie. deprotection, coupling, capping). After the last coupling step, the fully protected oligonucleotide is cleaved from the support and fully deprotected using a mixture of pyridine-2-carbaldoxime and tetramethylguanidine in dioxane/water.[36] This deprotection is allowed to occur at 37° C. for 20 h. After drying in vacuo, the oligonucleotide is purified by either HPLC or polyacrylamide gel electrophoresis (PAGE).

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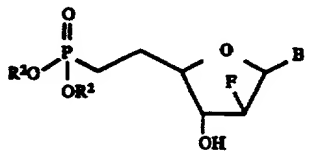
What is claimed is:

1. A compound having the formula:



wherein:

B is adenosine, N^6-benzoyladenine, thymine, guanine, or N^2-isobutyrylguanine; and

each R^2 is independently hydrogen, phenyl, alkyl (1–12C) or hydrogentriethylammonium ion.

2. The compound of claim 1 wherein B is guanine.

3. The compound of claim 2 wherein R^2 is hydrogen.

4. The compound of claim 3 wherein B is guanine.

5. The compound of claim 3 wherein B is N^2-isobutyrylguanine.

6. The compound of claim 3 wherein B is adenine.

7. The compound of claim 3 wherein B is N^6-benzoyladeaine.

8. The compound of claim 3 wherein B is thymine.

* * * * *

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Reissue Application of:

Buhr, *et al.*

U.S. Patent No.: 5,672,697 **Issued: September 30, 1997**

Serial No.: 652,978 **Group Art Unit: 1623 ***

Filing Date: February 8, 1991 **Examiner: G. Kunz ***

For: NUCLEOSIDE 5'-METHYLENE PHOSPHONATES

Assistant Commissioner for Patents
Washington DC 20231

Sir:

COMBINED REISSUE APPLICATION DECLARATION AND POWER OF ATTORNEY BY INVENTOR(S) OR ASSIGNEE[1]

(Complete A or B)

A. ☒ Declaration by Inventor(s):

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I verily believe that I am the original, first and sole inventor (if only one name is listed below), or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed in letters patent number **5,672,697** granted on **September 30, 1997** and in the foregoing specification and for which invention I solicit a reissue patent;

B. ☐ Declaration by Assignee:

* Enter the Group Art Unit and Examiner from which the original patent was issued.

[1] This declaration must be accompanied by Consent of Assignee for Reissue and Assignee's Statement of Ownership Interest.

Note: The assignee of the entire interest may make the declaration if the reissue application does not seek to enlarge the scope of the claims of the original patent, 37 C.F.R. §1.172.

@@ (Name of declarant), @@ (title), of @@ (Name of company or legal entity on whose behalf declarant is authorized to sign) declare that I am a citizen of @@ and resident of @@, that the entire title to letters patent number @@ for @@, granted on @@ to @@ is vested in @@ (name of company or legal entity), that I believe said named inventor(s) to be an original, first and sole inventor (if only one name is listed) or an original, first and part inventor (if plural names are listed) of the subject matter that is described and claimed in the aforesaid letters patent and in the foregoing specification and for which invention I solicit a reissue patent.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge the duty to disclose all information known to be material to the patentability of this application in accordance with 37 C.F.R. § 1.56.

☐ In compliance with this duty attached herewith is an Information Disclosure Statement in accordance with 37 C.F.R. § 1.97.

PRIORITY CLAIM

I hereby claim foreign priority benefit under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed.

☐ No such applications have been filed.

☐ Such applications have been filed as follows:

Country	Application No.	Date Filed	Priority Claimed
———	———	———	—
———	———	———	—
———	———	———	—

STATEMENT OF INOPERATIVENESS OR INVALIDITY OF ORIGINAL PATENT 37 C.F.R. §1.175

That I believe the original patent to be

☒ partly

☐ wholly

inoperative or invalid by reason of

☐ a defective ☐ specification, ☐ drawing, ☐ both

☒ said patent claiming

☐ more

☒ less

than patentee had a right to claim.

The scope of the claims of the original patent

☒ are enlarged

☐ are not enlarged

by this reissue application.[2]

In particular, patentees erred in not claiming the chemical compounds set forth in claims 9-26.

Note: Here, the Declarant must state at least one error in the original patent and describe it, e.g., "by reason of a defective specification or drawing" or "by reason of the patentee claiming more or less than he had the right to claim in the patent," particularly specifying the defects and distinctly specifying the excess or insufficiency in the claims. It is no longer required to specify details as to how the error arose or occurred. If any errors are corrected during reissue prosecution that were not specified herein, a Supplemental Reissue Declaration must be filed prior to allowance in compliance with 37 C.F.R. §1.175.

All errors being corrected in this reissue application up to the time of filing of this declaration under 35 CFR §1.175(a) arose without any deceptive intention on the part of the applicant.

OFFER TO SURRENDER ORIGINAL PATENT 37 C.F.R. §1.178

Applicant hereby offers to surrender the original patent, the reissue of which is sought herein.

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[2] 35 U.S.C. §251 forbids enlarging the scope of the original claims unless the reissue application is filed within two years of the grant of the original patent.

POWER OF ATTORNEY

I hereby appoint the following persons as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

John W. Caldwell	Registration No. 28, 937
Joseph Lucci	Registration No. 33,307

of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP,** One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 and

Herb Boswell	Registration No. 27,311
Laurel Bernstein	Registration No. 37,280
Edward D. Robinson	Registration No. 43,049
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Address all telephone calls and correspondence to:

Joseph Lucci
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: Chris Buhr	
Mailing Address: 870 Campus Drive #303	**Signature** **Date of Signature:** _____
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Name:	
Mailing Address:	**Signature** **Date of Signature:**
City/State of Actual Residence:	**Citizenship:**

Name:	
Mailing Address:	**Signature** **Date of Signature:**
City/State of Actual Residence:	**Citizenship:**

Note: Even though inventor(s) do not sign, complete above information for inventor(s).

☒ **By assignee or person authorized to sign on behalf of assignee:**

Date: 9-29-99

ISIS PHARMACEUTICALS, INC.

By: _____
B. Lynne Parshall, Esq.
Executive Vice President and
Chief Financial Officer

Check proper box(es) for any added page(s) forming a part of this declaration:

☐ Signature for fifth and subsequent joint inventors. Number of pages added ___.

☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added ___.

☐ Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 C.F.R. § 1.147. Number of pages added ___.

☐ Authorization of attorney(s) to accept and follow instructions from representative.

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR ISSUE APPLICATION FOR AUTHORIZATION OF ATTORNEY(S) TO ACCEPT AND FOLLOW INSTRUCTIONS FROM REPRESENTATIVE

The undersigned to this Combined Declaration and Power of Attorney hereby authorize(s) the U.S. Attorney(s) named herein to accept and follow instructions from:

_____
Name(s) of authorized representative(s)

_____
Address

_____

as to any actions to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney(s) and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorney(s) will be so notified by the undersigned.